Comparison of two commercial influenza RT-PCR assays by analyzing seasonal respiratory samples from India and the recommended influenza vaccine strains for the season 2019/2020 (recommended by WHO)

Karin Rottengatter[1]
[1]**altona Diagnostics GmbH, Hamburg, Germany**

Introduction

Influenza is an acute respiratory illness and imposes a considerable burden worldwide. Influenza affects the upper and/or lower respiratory tract and is caused by influenza virus, usually of type A or B. Influenza circulates continuously, causing seasonal epidemics in temperate regions and year-round epidemics in some tropical regions. Influenza A viruses may also cause pandemics characterized by rapid dissemination of a new, virulent influenza A subtypes to which there is little or no existing immunity [www.WHO.int].

Due to the constant changing of circulating influenza viruses, influenza vaccine composition is adapted annually (for both the Northern Hemisphere and the Southern Hemisphere) to more closely match currently circulating virus strains:

Recommended composition of influenza virus vaccines for the use in the 2019/2020 influenza season	
Northern Hemisphere*	**Southern Hemisphere****
A/Brisbane/02/2018 (H1N1) pdm09-like virus	A/Michigan/45/2015 (H1N1) pdm09-like virus
A/Kansas/14/2017 (H3N2)-like virus	A/Switzerland/8060/2017 (H3N2)-like virus
B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)	B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)
B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage)	B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage)

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Aim of the Study

The RealStar® Influenza Screen & Type RT-PCR Kit 4.0[a] (altona Diagnostics) is a CE-IVD marked assay for rapid detection and typing of influenza virus RNA (influenza A/B and swine flu (influenza A (H1N1) pdm09) within a single reaction for individual samples.

The Pandemic H1N1/09 Assay Set version 2.0 (Applied Biosystems™) contains a panel of primers and probes for use in TaqMan® RT-PCR assays for the *in vitro* qualitative detection and characterization of the 2009 pandemic H1N1 influenza virus in respiratory samples (Research Use Only). Four individual reactions have to be set up for each sample.

This study aims to evaluate the reliability of the two assays for the detection of the influenza virus strains recommended by the WHO for vaccination in the 2019/2020 season. In addition, 70 individual bronchial secretion samples from India collected during influenza season were analysed in parallel with both assays.

Material and Methods

Influenza virus strains which were recommended by WHO for vaccination for the season 2019/2020 were ordered at NIBSC (National Institute for Biological Standards and Control). Unfortunalety, influenza A virus Kansas/14/2017 (H3N2) was not available for testing.

NIBSC code	Influenza Reagent
17/264	A/Michigan/45/2015 (H1N1) pdm09-like virus
18/166	A/Switzerland/8060/2017 (H3N2)-like virus
17/196	A/Singapore/INFIMH-16-0019/2016 (H3N2)
17/254	B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)
17/248	B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage)

All sera were reconstituted as recommended in UTM™ Viral Transport Media (Copan). A pre-dilution (10E-5) was made for further testing.

From all five pre-diluted strains nucleic acids were extracted using the MagNA Pure 96 DNA and Viral NA Small Volume Kit on the MagNa Pure 96 System (Roche), following the protocol “Pathogen Universal 200 3.1”.

Real-time PCR was performed using the RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and with the RT-PCR Pandemic H1N1/09 Assay Set version 2.0 (Applied Biosystems) on the CFX96 (BioRad instrument).

A total of 70 individual bronchial secretion samples from patients with typical influenza symptoms were collected from individual patients in India during the influenza season 2018. The samples were previously tested and analysed with the RT-PCR Pandemic H1N1/09 Assay Set version 2.0. RNA was extracted as described above.

[a] Product not licensed with Health Canada and not FDA cleared or approved. Kits not available in all countries.

Results

Testing of influenza virus vaccine strains:

All five strains were detected and typed correctly according to the manufacturers claims. The RealStar® Influenza Screen & Type RT-PCR Kit 4.0 detected and typed all five influenza A and B strains. The RT-PCR Pandemic H1N1/09 Assay Set version 2.0 is designed only for detection of influenza A virus and detected and typed all of these correctly.

Table 1: RT-PCR results for influenza virus strains by RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and RT-PCR Pandemic H1N1/09 Assay Set version 2.0

Influenza Reagent	RealStar® Influenza Screen & Type RT-PCR Kit 4.0			RT-PCR Pandemic H1N1/09 Assay Set version 2.0*	
	Influenza A	Influenza B	(H1N1) pdm09	Influenza A	(H1N1) pdm09
A/Michigan/45/2015 (H1N1) pdm09-like virus	+	–	+	+	+
A/Switzerland/8060/2017 (H3N2)-like virus	+	–	–	+	–
A/Singapore/INFIMH-16-0019/2016 (H3N2)	+	–	–	+	–
B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage)	–	+	–	–	–
B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage)	–	+	–	–	–

* RT-PCR Pandemic H1N1/09 Assay Set version 2.0 does not detect influenza B

Testing of clinical samples (respiratory samples from the influenza season 2018) from India:

70 individual bronchial secretion samples from patients from India were pre-tested with the RT-PCR Pandemic H1N1/09 Assay Set version 2.0, and re-tested with RealStar® Influenza Screen & Type RT-PCR Kit 4.0. Results are shown in Table 2 and 3.

Table 2: Influenza-positive and -negative results by RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and RT-PCR Pandemic H1N1/09 Assay Set version 2.0

Total number of samples: 70		RealStar® Influenza Screen & Type RT-PCR Kit 4.0	
		Influenza positive	Influenza negative
RT-PCR Pandemic H1N1/09 Assay Set version 2.0	Influenza positive	46	0
	Influenza negative	2*	22

*2 out of 4 results were influenza B positive (not detectable by RT-PCR Pandemic H1N1/09 Assay Set version 2.0)

Table 3: Influenza A (H1N1) pdm09-positive and -negative results by RealStar® Influenza Screen & Type RT-PCR Kit 4.0 and RT-PCR Pandemic H1N1/09 Assay Set version 2.0

Total number of samples: 70		RealStar® Influenza Screen & Type RT-PCR Kit 4.0	
		(H1N1) pdm09 positive	(H1N1) pdm09 negative
RT-PCR Pandemic H1N1/09 Assay Set version 2.0	(H1N1) pdm09 positive	31	0
	(H1N1) pdm09 negative	1	38

Result Summary: The two assays show comparable results for the detection of influenza A (H1N1) pdm09 of both, vaccine strains for influenza A and the respiratory samples from India. Discordant results for (H1N1) pdm09 may be due to different sensitivities of the two assays. The Pandemic H1N/09 Assay Set version 2.0 does not detect influenza B and therefore missed 2 influenza B positives from the respiratory samples of India.

Conclusion

In countries with need for detection of influenza A, influenza A (H1N1) pdm09 and influenza B virus simultaneously, the RealStar® Influenza Screen & Type RT-PCR Kit 4.0 covers this demand with a high sensitivity and specificity. The advantage of detection and typing for all strains / types in one PCR reaction in comparison to four reactions by the Pandemic H1N/09 Assay Set version 2.0 leads to a faster and less error-prone result analysis with the RealStar® Influenza Screen & Type RT-PCR Kit 4.0.

Contact

Karin Rottengatter
altona Diagnostics GmbH
22767 Hamburg, Germany
info@altona-diagnostics.com
karin.rottengatter@altona-diagnostics.com